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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/28, 9/48, A91K 9/54, C12Q 1/04, 1/58, G01N 21/77 — A1

(11) International Publication Number: WO 95/11672

(43) International Publication Date: 4 May 1995 (04.05.95)

(21) International Application Number: PCT/US94/12332

(22) International Filing Date: 25 October 1994 (25.10.94)

(30) Priority Data:
08/142,600 28 October 1993 (28.10.93) US

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.

(54) Title: DETECTION OF H. PYLORI IN THE STOMACH

(57) Abstract

A method for the in vivo detection of urease-producing helicobacter in the upper stomach is disclosed. The dense carrier is divided into two separate groups which are combined with separate reagent indicators, one of which also contains urea. The carriers are food soluble products, preferably sugar beads having a diameter of approximately 0.2 to 3.0 mm. The treated carriers and urea are encapsulated in a soluble capsule which is administered to a patient. The density of the carriers cause the capsule to migrate to the gastric mucosa, where the capsule is dissolved, placing the reagents and urea in direct contact with the gastric mucosa. The urea reacts with any urease present in the stomach by creating ammonia, which increases the pH within the stomach. The two reagents react differently, through color change, to the increase in pH, which is viewed through use of an endoscope. A preferred first reagent is bromothymol blue (dibromothymolsulfonphthalein), which changes yellow in the presence of urease, and a preferred second reagent is phenol red (phenolsulfonphthalein) which turns red in the presence of urease.

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DETECTION OF H. PYLORI IN THE STOMACH

BACKGROUND OF THE INVENTION

Brief Description of the Invention

The instant invention relates to a novel method of in vivo diagnosis of upper gastrointestinal diseases.

Brief Description of the Prior Art

Factors adversely affecting the function of the gastrointestinal system in humans are exceedingly varied in their nature. Such disorders may arise in the upper or lower gastrointestinal tracts, or both. There is a broad range of causes of gastrointestinal disorders, including genetic, physiological, environmental and psychogenic factors. Accordingly, the diagnosis and management of these disorders can be exceptionally difficult.

Among the chronic disorders of the upper gastrointestinal tract are those which fall under the general categories of gastritis and peptic ulcer disease. The upper gastrointestinal tract is generally defined as including the esophagus, the stomach, the duodenum, the jejunum and ileum. Peptic ulcers are lesions of the gastrointestinal tract lining, characterized by loss of tissue due to the action of digestive acids and pepsin. It has generally been held that peptic ulcers are caused by gastric hypersecretion, decreased resistance of the gastric lining to digestive acids and pepsin, or both. Gastritis is, by definition, an inflammation of the stomach mucosa. In practice, though, the disorder is manifested by a broad range of poorly-defined, and heretofore inadequately treated, symptoms such as indigestion, "heart burn", dyspepsia, and excessive eructation.

As with the management of any disorder, the rapid, precise, and accurate diagnosis of gastrointestinal disorders is of paramount importance. The typical means used to diagnose the gastrointestinal disorder presented by a given patient will depend upon such factors as the nature and severity of symptoms, the overall health of the individual, the medical history of the patient, the need for a specific diagnosis in order to implement a treatment with reasonable likelihood of success, and the availability of diagnostic devices. However, the diagnostic methods typically employed in the art are often slow, cumbersome, costly, and may yield equivocal or inaccurate results. Thus, for patients not having severe symptoms, a precise diagnosis of a gastrointestinal

disorder might not be attempted. Such patients may simply be treated with conventional therapies, such as with antacids or drugs which inhibit stomach acid secretion. While such therapies might provide temporary symptomatic relief, a cure is often not effected. More effective treatments may depend upon better diagnoses of the actual underlying gastrointestinal disorder. In particular, it has been discovered that many such gastrointestinal disorders are mediated by infection of gastric mucosa by Helicobacter pylori. H. pylori is a Gram-negative spiral organism which produces the enzyme urease. The organism is predominantly found beneath the mucus layer of the luminal aspect of the gastric epithelium and in the gastric pits. Helicobacter can be diagnosed by blood test for antibodies, breath test, or biopsy of the stomach lining. Antibodies, however, can remain positive for many months after the bacteria have been eradicated. The presence of antibodies presents a falsely positive result in approximately 10 to 15% of patients. Biopsies are relatively quick; however, they add time, expense and risk. Although relatively minor, there is a 1 in 20,000 risk of bleeding from a biopsy site. Biopsies cannot be performed on patients who have a tendency to bleed, such as patients with hemophilia and liver disease. Additionally, it has recently been found that helicobacter is patchy, thereby requiring multiple biopsies to obtain 100% accuracy. The cost for a biopsy is approximately $100. Biopsies also increase the risk of the person handling the tissue being exposed to HIV. If a urease test is used, the biopsy sample must be placed in the test by the nurse, thereby requiring an additional person during the test.

The prior art has disclosed testing for gastrointestinal disorders, the majority of which have been in vitro. Many tests have also been disclosed using urea and indicators.

Marshall, 4,748,113 discloses compositions and methods for the diagnosis of gastrointestinal disorders involving urease. Methods include obtaining a gastric sample material and contacting the material with a composition including urease and an indicator.

Marshall 4,830,010, discloses methods for the diagnosis of gastrointestinal disorders. The method steps include administration of urea-containing compositions prior to assay.

Steward et al, 5,139,934 disclose substrate compositions and method of urease assay. The method is an in vitro immunoassay that includes the use of pH indicators.

Nagatsu et al, 4,147,692 disclose methods and compositions for measuring enzymatic activities and correlating such activities with various disease states.

Kraffczyk et al, 3,873,369 disclose colorimetric indicators for the determination of urea.

Vasquez et al, 4,851,209 disclose in vivo diagnostic procedures for the clinical evaluation of gastrointestinal ulcer disease using radioactive isotopes. Procedures involve prior administration of a diagnostic pharmaceutical followed by scintigraphic imaging of the gastrointestinal area of interest with scintigraphic imaging equipment.

Although the use of urease or other indicators has been used in combination with pH indicators, all except Vasquez et al are conducted in vitro.

The instant invention discloses a method of detecting the alkaline pH change in vivo. The test dramatically cuts down the number of biopsies required and is safe for patients having any bleeding tendencies while being rapid and low cost. Additionally, through the color change, it can be determined if the change is a true positive or a false positive reaction.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages of the instant disclosure will become more apparent when read with the specification and the drawings, wherein:

FIGURE 1 illustrates the location of the beads in the stomach and the urea/ammonia transfer.

SUMMARY OF THE INVENTION

A method for the in vivo detection of urease-producing helicobacter in the upper stomach is disclosed. A dense carrier is used which is divided into two separate groups, the first combined with a first reagent indicator and the second combined with a second reagent indicator and urea, i.e. ^{14}C-Urea or ^{13}C-Urea. The carriers are food soluble products, preferably sugar beads having a diameter of approximately 0.2 to 3.0 mm. The carrier and reagent can be combined through coating the carrier or combining the carrier and reagent. The treated carriers and urea are encapsu-

lated in a soluble capsule which is administered to a patient. A buffer can be added, if desired, to obtain specific results. The density of the carriers cause the capsule to migrate to the gastric mucosa. The gastric juices dissolve the capsule containing the reagents and urea thereby placing the two reagents and urea combination in direct contact with the gastric mucosa. The urea reacts with any urease present in the stomach by creating ammonia, which causes the pH within the stomach to increase. The two reagents react differently, through color change, to the increase in pH, which is viewed through use of an endoscope. A preferred first reagent is bromothymol blue (dibromothymolsulfonphthalein), which changes yellow in the presence of urease, and a preferred second reagent is phenol red (phenolsulfonphthalein), which turns red in the presence of urease.

DETAILED DESCRIPTION OF THE INVENTION

The instant disclosure uses indicators to detect alkaline pH change in the stomach during endoscopy. A change in the colors of the indicators detects pH change within the stomach. A certain combination of colors will indicate the presence of helicobacter, or H. pylori, organisms.

Urea has the formula H_2NCONH_2 and is a naturally occurring product of protein metabolism. Gastric materials from humans or other animals having gastrointestinal disorders contain relatively large quantities of urease (urea aminohydrolase), which hydrolizes urea to ammonium carbonate or ammonia and carbon dioxide. Normally urease is present in the body in only trace amounts, performing the function of decomposing urea. H. pylori increases the amount of urease above normal in the affected areas. The increased urease reacts with the administered urea by creating ammonia, which causes an indicator color change due to the increased alkalinity.

The indicators useful in this invention are weak acids with sharply different colors in their dissociated (ionized) and un-dissociated (neutral) states. The indicators useful herein have pK_a values of from about 6.5 to about 8.5, preferably from about 7.0 to about 8.0. The color exhibited by the indicator in the present composition will depend upon the pH of the composition, the particular indicator used, and the dissociation constant (K_a) for that indicator (i.e., pK_a $|\log_{10}K_a$). As the color exhibited by the indicator changes over a

range of pH values (pH = $\log_{10}$ [H+]), the indicators useful in the present compositions chang color over a pH range of from about 5.5 to about 9.0, preferably from about 6.5 to about 8.5. The pH of the present composition are accordingly adjusted to a pH at least about one pH unit lower than the pK_a of the indicator used (i.e. having a hydrogen ion concentration $[H^+]$ ten times less than (10% of) the hydrogen ion concentration in a solution having a pH equal to the pK_a of the indicator). Preferably, the pH is adjusted to a pH about two pH units below the pK_a of the indicator. Adjustment of the pH of the present compositions can be effected by addition of a base (e.g. sodium hydroxide) or an acid (e.g. hydrochloric acid or citric acid). Thus, preferably, the pH of the composition of this invention is adjusted to a pH of from about 5.0 to about 6.5, with the preferred embodiment being from about 5.0 to about 6.0.

The preferred reagents are bromothymol blue (dibromothymolsulfonphthalein) indicator, Reagent 1, and phenol red (phenolsulfonphthalein) indicator, Reagent 2. Other indicators useful herein include p-nitrophenol, neutral red (2-methyl-3-amino-6-dimethylaminophenazine), quinoline blue (cyanine), cresol red (o-cresolsulfonphthalein), and thymol blue (thymolsulfonphthalein). Indicators among those useful herein are described in the The Merk Index (9th ed. 1976), incorporated by reference herein. Reagent 2 has urea added to react to the urease enzyme, if present. The urea penetrates the mucus layer of the stomach to come into contact with the urease-containing bacteria, H. pylori, on the stomach wall. The urea/urease combination creates ammonia which migrates outward through the mucus layer to come into contact with the Reagents.

The urea is added to a soluble, dense carrier at approximately 1-20 grams per kilogram of carrier. The preferred carrier is beads, such as non pareil beads, although any dense carrier can be used which has sufficient density to carry the capsule to the stomach mucosa. In the preferred embodiment the Reagents 1 and 2 are put into the stomach in a solid phase, such as beads, which can be individually identified in the stomach. The reagents should be coated onto small diameter beads, preferably 0.2-3.0 mm, with the preferred size being approximately 2 mm. The 2 mm. size of the beads provides the advantages of visibility as well as preventing obstruction of the endoscope in the event not all of the beads dissolve. A suitable method of making such beads would be

to use sugar beads, such as non pareil seeds, with a mesh size of 25-35. The non pareil beads provide the density required to migrate to the mucosa, either in the capsule or after the capsule dissolves. A less dense vehicle, which floats within the gastric juices, would prevent the Reagents from being placed onto the mucosa. U.S. Patent No. 3,121,041, issued to Stern et al, discloses the use of a plug, impregnated with a radioactive material, in combination with a soluble capsule. The spongy plug disclosed in Stern would float within the gastric juices, providing several disadvantages. In order to obtain the contrasting results of the two reagents, two impregnated sponges must be used within the capsule, thereby increasing manufacturing expenses. The Stern et al patent discloses tapping the sponges into the capsule. The use of two sponges would possibly double the time required to produce the Stern capsule. Additionally, as the sponges would float within the gastric juices, the Reagents would be diluted and possibly affected by the contents of the gastric juices. The Reagents must be placed directly onto the mucosa to allow the urea to migrate to the stomach wall, react to the urease created by the H. pylori, create ammonia, and subsequently alter the pH. To allow for a dilution factor would require increasing the amount of urea used in the capsule. The goal of the instant disclosure, as well as other disclosures relating to the use of radioactive materials, is to reduce the quantity administered to the patient. By placing the urea directly onto the mucosa, dilution is reduced to a minimum and therefore a small quantity produces superior accuracy. The beads cannot be coated as commonly known in the time release capsule art, as the reagents on all the beads must be activated simultaneously to obtain a reliable reading. U. S. Patent 3,383,283 to Brindamour discloses time release beads coated with a fatty acid. The fatty acid coating, along with many other coatings, would cause all or some of the beads to float within the gastric juices, again preventing contact with the mucosa.

The disclosed testing procedure is performed in vivo, thereby frequently eliminating the need for a biopsy. In order to view the reagent color change, the beads must remain in a single area. To accomplish this, the beads must not float, but rather lie directly on the mucosa, at the source of the bacteria. It has recently been discovered that H. pylori within the stomach is not continuous or in large areas, but rather patchy within the stomach wall. In the instant disclosure,

the natural dispersal of the beads nto the mucosa cover a sufficient areas to react with at least one area of H. pylori bacteria. Any floating indicators which come in contact with the mucosa on either a temporary or scattered basis, have a narrow chance to come in direct contact with the affected area.

Beads which do not dissolve after a few minutes in the stomach can cause an obstruction of the endoscope if they are below the preferred size. Other types of dense vehicles can be used as long as they are capable of absorbing the required reagents and of dissolving within a few minutes. When using a powdered carrier, the reagents are mixed with the carrier, the carrier is allowed to dry, and, if necessary, re-ground to powder form. The beads have the advantage that coating the beads with the reagents is a simpler, more economical method of obtaining optimum results.

An example of manufacture of the beads would be:

Reagent 1 - bromothymol blue indicator
buffer (pH=6.0)
sugar beads

Reagent 2 - phenol red indicator
buffer (pH=6.0)
sugar beads
urea

The beads are preferably encapsulated into a quick-dissolving gelatin capsule for delivery to the stomach in mass and undiluted. The capsule can be swallowed with a small amount of liquid, such as water, to more rapidly deliver the capsule and speed the dissolving of the capsule. If necessary, a buffer, such as citrate, having a pH between 4.0 and 6.0 can be added to the liquid to render the gastric pH initially slightly acid. Reagents applied in liquid form will mix with each other, even if taken separately, providing an indefinite result.

Additional ingredients can be added with the reagents to produce any specific desired results. An example of this would be to buffer an Acid pH with a stable buffer such as citrate buffer at pH 6.0, 30 mis. The buffer can be added to the seed-coating along with the reagents or can be placed in powdered form in the capsule. The use of a buffer adds stability to the shelf life of the capsules.

In Figure 1 the stomach wall, bacteria with urease, and mucus layers are shown with the reagent beads resting on the mucus layer. As the urea released from the Reagent 2 comes in contact with the urease, ammonia is generated. The ammonia rises through the mucus layer and comes into contact with the Reagent indicators, causing an increase in the pH and the Reagents to change color.

To administer the test, the subject takes one to two capsules with 30 mis of pH 6.0 buffer immediately before endoscopy. It takes approximately 5 minutes for the endoscope to reach the stomach, at which time the capsules have dissolved and the granules are resting and slowly dissolving on the surface of the gastric mucosa. Through the endoscope, the examining person can detect the color changes of the reagents, if any, which indicate the presence of the helicobacter organisms.

In the following example Reagent 2 is yellow at acid pH, changing to red at alkaline pH and Reagent 1 is yellow at acid pH, changing to blue at alkaline pH. The instant invention relies on a differential color change to identify a true positive from a false positive reaction. It is the differential which is of importance, not the colors themselves and any colors and/or reagents specifically used herein are examples and in no way limit the scope of the invention.

READING EXAMPLE I

Negative result, (no urease, stomach is acid)

Regent 1 (yellow)		Both remain yellow
	no urease	
Reagent 2 (yellow)		no pH change occurs

READING EXAMPLE II

False positive result (stomach has an alkaline pH; for example, bile is in stomach or patient salivates excessively)

Reagent 1 (yellow) Changes to blue

no urease, $pH > 6.5$

Reagent 2 (yellow) Changes to red

READING EXAMPLE III

True positive result (stomach is acid but contains urease)

Reagent 1 (yellow) urease Remains yellow

$pH < 6$ no pH change occurs.

Reagent 2 (yellow) urease Changes red

pH rises > 6.5

The presence of red and yellow reagent, but not blue reagent, indicates that urease is in the stomach (i.e. Helicobacter).

What is Claimed is:

1. The method of in vivo detection of urease-producing helicobacter in the upper stomach comprising the steps of:

obtaining at least two separate groups of dense carriers;

combining the first of said at least two separate groups of dense carriers with a first reagent indicator;

combining the second of said at least two separate groups of dense carriers with a combination of a second reagent indicator and urea;

encapsulating said first reagent and said second reagent-urea combination in a soluble capsule;

administering said capsule to a patient;

causing said capsule to migrate to the gastric mucosa through the density of said carriers,

dissolving said capsule containing said first reagent and said second reagent-urea combination in the gastric juices;

wherein said first reagent and said second reagent-urea combination are placed in direct contact with the gastric mucosa, allowing said urea to react with any urease present in the stomach thereby creating ammonia, said ammonia causing the pH within said stomach to increase, thereby causing said first reagent and said second reagent to react to said increase in pH, said reaction being viewed through endoscopy.

2. The method of Claim 1 wherein said dense carriers are soluble food products.

3. The method of Claim 2 wherein said food products are sugar beads.

4. The method of Claim 3 wherein said sugar beads have a diameter of approximately 0.2 to 3.0 mm.

5. The method of Claim 1 wherein said first reagent indicator and said second reagent indicator react through color change based on the pH present in said stomach.

6. The method of Claim 5 wherein said first reagent and said second reagent change to colors different from one another.

7. The method of Claim 1 wherein said first reagent is a bromothymol blue (dibromothymolsulfonphthalein) indicator, and said second reagent is a phenol red (phenolsulfonphthalein) indicator.

8. The method of Claim 1 wherein said combination of said dense carrier and said reagent is through coating said carrier with said reagent.

9. The method of Claim 1 wherein said combination of said dense carrier and said reagent is through said carrier absorbing said reagent.

10. The method of Claim 1 wherein said urea is ^{14}C-Urea.

11. The method of Claim 1 wherein said urea is ^{13}C-Urea.

12. The method of Claim 1 wherein a buffer is added to said combination of said dense carrier and said reagents.

13. A diagnostic device for detection of urease-producing helicobacter within the stomach of a human or lower animal subject comprising:

a first dense carrier material, the density of said carrier being sufficient to cause said carrier to migrate to the gastric mucosa;

a second dense carrier material, the density of said carrier being sufficient to cause said carrier to migrate to the gastric mucosa;

a first reagent, said first reagent being in contact with said first dense carrier;

a second reagent, said second reagent being in contact with said second dense carrier;

urea, said urea being in contact with said second dense carrier material;

a soluble capsule, said soluble capsule being soluble in the gastric fluids containing said first carrier and said second carrier.

14. The diagnostic device of Claim 13 wherein said first dense carrier material and said second dense carrier material are food products.

15. The diagnostic device of Claim 14 wherein said first dense carrier material and said second dense carrier material are beads.

16. The diagnostic device of Claim 13 wherein said contact between said first dense carrier and said first reagent is through coating said carrier with said reagent.

17. The diagnostic device of Claim 13 wherein said contact between said second dense carrier and said second reagent and said urea is through coating said carrier with said reagent and urea.

18. The diagnostic device of Claim 13 wherein said contact between said first dense carrier and said first reagent is through mixing said carrier with said reagent and allowing the combination to dry.

19. The diagnostic device of Claim 13 wherein said contact between said second dense carrier and said second reagent and said urea is through mixing said carrier with said reagent and said urea and allowing the combination to dry.

20. The diagnostic device of Claim 13 wherein said urea is ^{14}C-Urea.

21. The diagnostic device of Claim 13 wherein said urea is ^{13}C-Urea.

22. The method for the diagnosis in a subject of the presence of urease producing helicobacter comprising the steps of:

a- administering to said subject a safe and effective amount of urea, said urea being carried by a dense vehicle, a first portion of said dense vehicle being in combination with a first reagent and a second portion of said dense vehicle being in combination with a second reagent, said vehicle, said reagents and said urea being encapsulated in a capsule, said capsule being soluble in gastrointestinal fluids,

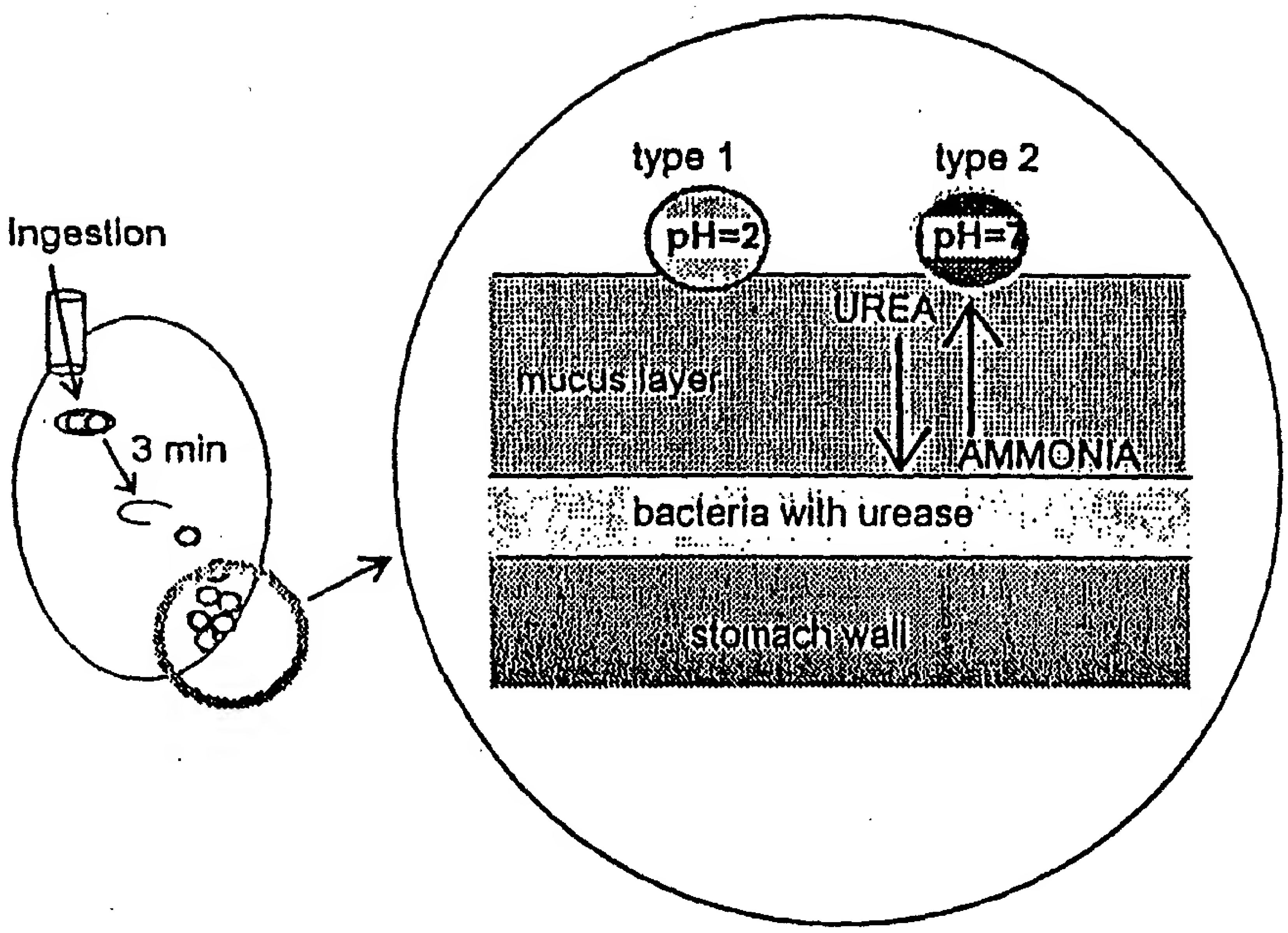
b- drinking approximately 10 ml of water,

c- delivering said capsule through gravity to the gastric mucosa,

d- dissolving said capsule in the gastric juices thereby allowing said reagent/vehicle combination and said urea to be in direct contact with said gastric mucosa,

e- causing the urea to migrate to the stomach wall and reacting with any urease present, wherein said reaction with said urease produces ammonia, thereby altering the pH within the stomach,

f- viewing said first reagent and said second reagent through endoscopy for color change, wherein the color change of the first reagent in relation to the color change of the second reagent indicate the presence of urease producing heliobacter within the stomach.


Ingestion
3 min
type 1
type 2
pH=2
pH=7
UREA
mucus layer
AMMONIA
bacteria with urease
stomach wall

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12332

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/28, 9/48, 9/54; C12Q 1/04, 1/58; G01N 21/77
US CL :424/451, 453, 458, 474, 490; 435/12, 34, 288; 436/169, 811
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/451, 453, 458, 474, 490; 435/12, 34, 288; 436/169, 811

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, DERWENT, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,262,156 (ALEMOHAMMAD) 16 NOVEMBER 1993, see entire document.	1-22
A,P	US, A, 5,314,804 (BOGUSLASKI ET AL.) 24 MAY 1994, see entire document.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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